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Cord blood (CB) transplantation in adults is limited by low cell dose. This leads to delayed engraftment and delayed immune reconstitution with susceptibility to infections a major cause of morbidity and mortality. We hypothesize that the infusion of ex vivo CB T cells expanded using an anti-CD3/anti-CD28 bead may provide more rapid immune reconstitution, thereby reducing infectious complications. Prior to initiating a clinical trial, conditions for ex vivo CB T cell expansion need to be optimized. Variables investigated include addition of N-acetylcysteine (NAC) to culture (potentially reducing apoptosis), optimizing levels of interleukin 2 (IL-2), and continuous addition of anti-CD3/anti-CD28 beads to maintain a bead to cell ratio of 3:1. **Methods:** CB T cells were isolated from CB sample using anti-CD3/anti-CD28 paramagnetic beads and co-cultured with beads for a total of 14–28 days. Comparisons included: (1) 0 versus 10 mM NAC; (2) maintained 3:1 bead:cell ratio versus dilution with culture; (3) IL-2 concentration (0, 20, 200, 2000, and 20,000 IU/mL). Phenotypes were investigated using flow cytometry and T cell receptor diversity measured by spectratyping. **Results:** Overall, 300–1200-fold increases in T cell numbers were achieved. (1) While NAC did not significantly impact cell numbers, flow cytometry suggested cell death was reduced. (2) Maintaining a constant bead to cell ratio throughout culture did not improve proliferation. (3) Greatest T cell proliferation was obtained when 2000 and 20,000 IU/mL IL-2 were used. At the time of maximal expansion, the majority of the $CD4^+$ cells retained a naive phenotype ($CD27^+CD28^+$). A small proportion of late stage "effector" cells were present ($CD27^-CD28^-$ or $CCR7^-CD45RA^+$). $CD8^+$ T cell differentiation toward a "memory" phenotype ($CD27^+CD28^-$ or $CD27^-CD28^+$, or $CCR7^-CD45RA^-$ or $CCR7^+CD45RA^-$) was observed. Significantly, the expanded CB T cells retained a polyclonal TCR diversity. **Conclusions:** These preliminary data suggest that the ex vivo expansion of CB T cells may yield sufficient numbers of T cells to potentially improve immune reconstitution in CB recipients and reduce post-transplant mortality. Studies assessing the functionality of the expanded T cells are currently underway.

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ADOPTIVE TRANSFER OF IN VITRO GENERATED T CELL PRECURSORS IMPROVES T CELL RECONSTITUTION AND MEDIATES GRAFT-VERSUS-TUMOR ACTIVITY WITHOUT GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

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Immunoincompetence after allogeneic hematopoietic stem cell transplantation (HSCT) particularly affects the T cell lineage resulting in significant morbidity and mortality from opportunistic infections. Recent studies have shown that murine T cells and their precursors can be generated from hematopoietic stem cells (HSC) in vitro using OP9 bone marrow stromal cells (H2Kk/H2Kb) expressing the Notch 1 ligand Delta-like 1 and growth factors. In this study we determined the effects of adoptively transferred in vitro generated T cell precursors on T cell reconstitution after allogeneic HSCT. We selected HSC (Lin-Sca-1hi c-kit+) from mouse bone marrow (BM) of various strains and cultured these cells on a monolayer of OP9-DL1 cells in the presence of growth factors. T lineage cell development proved to be strain independent and is therefore not MHC restricted in this culture system. C57BL/6 dervied HSC expanded 2000–5000-fold within 3–4 weeks and consisted of 90–95% CD4-CD8-double negative (DN) T cell precursors after 16–28 days of culture. We infused these cells (8×10^6) with T cell depleted (TCD) BM (5×10^6) or purified HSC into allogeneic recipients using minor antigen mismatched and MHC class I/II mismatched transplant models. Progeny of OP9-DL1 derived T cell precursors were found in the thymus and the periphery significantly improving thymic cellularity, and thymic and splenic donor T cell chimerism. Combination of T cell precursor administration and pre-conditioning with keratinocyte growth factor (KGF) further improved thymic engraftment of OP9-DL1 derived T cell precursors. T cell receptor repertoire and proliferative response to foreign antigen of OP9-DL1 derived mature T cells were intact. Moreover, Th1-type cytokine secretion of OP9-DL1 derived splenic T cells after stimulation with PMA/ionomycin was better than that of BM or HSC derived donor T cells. Progeny of OP9-DL1-derived T cell precursors remained detectable for at least 60 days after transplant with intact cytokine production. Administration of in vitro generated T cell precursors did not induce GVHD but mediated significant graft-versus-tumor (GVT) activity (determined by in vivo bioluminescence imaging) resulting in a subsequent significant survival benefit. We conclude that the adoptive transfer of OP9-DL1 derived T cell precursors significantly enhances donor T cell reconstitution in allogeneic HSCT recipients and results in GVT activity in the absence of GVHD.

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RECONSTITUTION OF IMMUNITY TO ADENOVIRUS (Ad) AFTER PEDIATRIC BONE MARROW TRANSPLANT

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Adenovirus is a significant cause of morbidity and mortality in pediatric HSCT patients. Severity of immune suppression appears to be the most important risk factor for serious Ad disease. Without proven antiviral therapy, cellular immunity is the most effective protection against Ad disease. To better understand Ad immune recovery post-BMT, we prospectively monitored viral load, Ad subtype infection rates, and recovery of Ad cellular immunity in 22 pediatric HSCT patients (ages 14 months–20 years) who received matched-related (MRD n = 6), mismatched related (haplo n = 6), or unrelated donor (MUD n = 10) grafts. Real-time PCR (Q-PCR) provided quantitative measure of Ad viral load and detected Ad in blood samples from 68% (15/22) of patients on at least one occasion post SCT. 2/6 MRD recipients had Ad detected in their blood vs 8/10 MUD and 5/6 haplo patients. 13/16 (81%) of patients who provided stool samples were +ve for Ad on at least one occasion. Ad subgroup C predominated in blood (93%), while subgroups varied in stool: A(62%), B(12%), C(62%), D(6%), E(6%), F/G(44%). 8/16 patients were positive for multiple subtypes in their stool. Mean Ad-specific spot forming cells (SFC)/1×10^6 PBMC as measured by IFNg ELISPOT assay was not significantly different in the MRD and MUD groups at 3, 6, and 12 months post HSCT ($P \geq .1$). At 3 months, MUD patients had mean 29 SFC/10^6cells (range: 0–190) versus 27 SFC/10^6 cells (range: 0–100) in the MRD group. By 12months, MUD patients had 63 (0–225) versus 78(17–201) SFC/10^6 cells in MRD group. However, immune response to adenovirus was significantly delayed ($P = .005$) in Haplo patients at 3months (4 SFC/10^6cells, range:0–18) and 12months (23 SFC/10^6cells, range:0–60). Mortality from adenovirus was 4.5% (1/22). Mean ALC in MUD, MRD, and Haplo recipients at 12 months were:1901/ul (772–3728), 2153/ul (948–3280), 1713/ul (1128–2406), respectively ($P > .1$). Mean CD4 and CD8 counts/μl in MUD, MRD, and Haplo recipients at 12months were: 835 (range:37–1656) and 589 (range:18–2404), 1373 (560–2169) and 530 (332–706), and 1055 (451– 1659) and 398 (178–620), respectively ($P > .1$). In conclusion, our data shows recipients of T cell depleted HSCT products from haploidentical donors have significantly delayed Ad-specific cellular immune recovery. Further, ALC does not predict specific immune recovery to adenovirus. We have initiated a clinical study of donor-derived adenovirus-specific CTL infusions for the prophylaxis and treatment of Ad infection post SCT that will target this patient population.